7 SHEETS CLMS

CLAIMS

1. Method of identifying a modulator of CD28 comprising comparing a structural model of a candidate modulator with a structural model of CD28 to thereby determine whether the modulator will bind to CD28, wherein the structural model is derived from, or comprises, structural coordinates of a crystal of: (i) CD28, (ii) a fragment of CD28, or (iii) a homologue of (i) or (ii).

2. Method according to claim 1 wherein said comparison comprises fitting (docking) the structural model of the candidate modulator with the structural model of CD28, and optionally determining the binding free energy of binding between the candidate modulator and CD28, wherein a low (more negative) binding free energy indicates that the candidate is likely to bind to CD28.

3. Method according to claim 2 wherein the binding free energy is calculated by (i) summing the free energies of interatomic contacts between the structural model of the candidate modulator and the structural model of CD28, or
(ii) determining the free binding energy between the force field of the candidate modulator and the force field of CD28.

4. Method according to any one of the preceding claims wherein whether or not the candidate modulator binds to CD28 comprises comparing the fitting of the structural model of the candidate modulator and the structural model of CD28 with the fitting of a structural model of another protein bound to a ligand, to thereby determine whether or not the candidate modulator will bind to CD28.

5. Use of the structural coordinates of a crystal of (i) CD28, (ii) a fragment of CD28, or (iii) a homologue of (i) or (ii), to identify a modulator of CD28.

6. Method or use according to any one of the preceding claims wherein the structural coordinates are obtainable by subjecting a crystal of (i) CD28, (ii) a

fragment of CD28, or (iii) a homologue of (i) or (ii), to X-ray diffraction measurements and deducing the structural coordinates from the diffraction measurements.

7. Method or use according to any one of the preceding claims wherein the crystal is of (i), (ii) or (iii) bound to a CD28 specific antibody or a fragment of said antibody.

8. Method or use according to any one of the preceding claims wherein the crystal has the structural coordinates shown in Table 4.

9. Method or use according to any one of the preceding claims which further comprises contacting the identified modulator of CD28 with (i) CD28, (ii) a fragment of CD28, or (iii) a homologue of CD28 or the fragment, to determine whether or not the modulator is capable of binding, or modulating the activity of, CD28.

10. A crystal as defined in any one of claims 1, 7 or 8.

11. Method of making a crystal as defined in any one of claims 1, 7 or 8 comprising providing a solution that comprises (i) CD28, (ii) a fragment of CD28, or (iii) a homologue of (i) or (ii), and optionally a CD28 specific antibody or fragment of said antibody, and subjecting the solution to conditions that cause the crystal to form.

12. Method according to claim 11 comprising:

(a) expressing (i), (ii) or (iii) in the form of a fusion protein with a second protein that is able to form a homodimer, wherein the presence of the second protein in the fusion protein causes (i), (ii) or (iii) to dimerise,

(b) cleaving the second protein from the fusion protein,

(c) reducing and alkylating one or more of the disulphide bonds present in the stalk-like region of (i), (ii) or (iii), and

(d) crystallising (i), (ii) or (iii) bound to a Fab fragment of an antibody.

13. Method according to 12 wherein the second protein mentioned in step (b) is an Fc fragment of an antibody.

14. Method according to any one of claims 11 to 13 wherein prior to crystallisation (i), (ii) or (iii) is expressed in the form of a fusion protein with an Fc fragment of an antibody, and optionally (i), (ii) or (iii) is cleaved from the fusion protein by thrombin.

15. Method according to any one of claims 11 to 14 wherein the (i), (ii) or (iii) is present in monomeric form in the crystal and/or one or more cysteine residues in the stalk-like region of (i), (ii) or (iii) are ethylated in the crystal.

16. A machine-readable data storage medium comprising a data storage material encoded with a machine readable data which when read by an appropriate machine is capable of displaying a representation of a crystal as defined in claim 1, 7 or 8.

17. A computer program comprising program code means for performing the method or use of any one of claims 1 to 9 when said program is run on a computer.

18. A computer program product comprising program code means stored on a computer readable storage medium for performing the method or use of any one of claims 1 to 9 when the said program product is run on a computer.

19. An antibody that induces superagonistic signalling by a cell surface receptor, wherein said antibody binds to the extracellular portion of the receptor at a membrane proximal region and said receptor comprises a cytoplasmic domain which is dependent on an extrinsic protein kinase, wherein said antibody does not bind only the C'-D loop of human CD28.

20. A chimeric protein that induces superagonistic signalling by a cell surface receptor, which chimeric protein comprises

(i) sequence representing a fragment of a ligand of the receptor, or a homologue of such a fragment, wherein the fragment or homologue is capable of binding to the extracellular portion of the receptor at a membrane proximal region, and
(ii) an Fc region of an antibody,
wherein said receptor comprises a cytoplasmic domain which is dependent on an extrinsic protein kinase.

21. A chimeric protein that induces superagonistic signalling by one or two types of cell surface receptor, which chimeric protein comprises two Fv regions of an antibody that may be the same or different, wherein at least one of the Fv regions is capable of binding to a first receptor, and the other Fv region either binds to (i) said first receptor, or (ii) a second type of cell surface receptor which is found on a cell that contacts a cell which expresses (i), wherein said first receptor, and optionally also said second receptor, comprises a cytoplasmic domain which is dependent on an extrinsic protein kinase.

22. An antibody or chimeric protein according to any one of claims 19 to 21 which
(i) binds orthogonally to the main axis of the domain of the receptor which it is binding, and/or
(ii) which lies parallel to the cell surface when bound to the receptor, and/or
(iii) which binds to a β-strand polypeptide chain of the receptor, and/or
(iv) which binds within 75Å of the cell surface.

23. An antibody or chimeric protein according to any one of claims 19 to 22 which binds to a sequence as shown in Table 1 or an equivalent homologous sequence in the proximal membrane region of a receptor which is capable of being induced to signal by the antibody or chimeric protein.

24. An antibody or chimeric protein according to any one of claims 19 to 23 wherein said receptor
(i) comprises an ITAM motif, ITIM motif or "switch" signalling motif, and/or

(ii) is a member of the CD28 family of proteins, and/or

(iii) is expressed on the surface of a cell of the immune system, and/or

(iv) comprises a cytoplasmic domain capable of being phosphorylated by a Src kinase, and/or

(v) comprises a cytoplasmic domain capable of being dephosphorylated by CD45, and/or

(vi) is one of the receptors listed in Table 2, or is a homologue thereof.

25. Method of obtaining an agent capable of inducing superagonistic signalling by a receptor as defined in claim 20 or 24, comprising determining whether a candidate agent binds to a membrane proximal extracellular region of the receptor, to thereby determine whether the candidate agent is capable of superagonising the receptor.

26. Method according to claim 25 comprising determining whether a candidate agent which binds to the receptor, fails to bind to a mutated version of the receptor wherein one or more amino acids in a membrane proximal extracellular region of the receptor have been mutated, failure to bind to the mutant receptor indicating that the agent is capable of inducing superagonistic signalling by the receptor.

27. Method according to claim 25 or 26 wherein the method is performed by contacting the candidate agent with (i) a full length receptor with said mutations, or (ii) a homologue of (i) with said mutations, or (iii) a fragment of (i) or (ii) comprising said mutations.

28. Method according to claim 25 wherein the location of the binding of the candidate agent is determined by contacting the candidate agent with a peptide which comprises sequence from a membrane proximal extracellular region of the receptor and determining whether the candidate agent binds to the peptide, the binding of the candidate agent to a peptide indicating that the agent is capable of inducing superagonistic signalling by the receptor, wherein said sequence is at least 5 amino acids in length.

29. Method according to claim 28 comprising contacting the candidate agent with an array of overlapping peptides, which peptides represent fragments of the receptor and are 5 to 20 amino acids in length, the binding of the candidate agent to a peptide which represents a membrane proximal extracellular region of the receptor indicating that the agent is capable of inducing superagonistic signalling by the receptor.

30. Method of obtaining a superagonistic antibody as defined in any one of claims 19 or 22 to 24 comprising

(i) screening antibodies for the ability to induce superagonistic signalling by a receptor according to claim 20 or 24, wherein said antibodies have been obtained by immunizing an animal with (a) said receptor, (b) a homologue of said receptor, or (c) a fragment of (a) or (b), or

(ii) screening antibodies for the ability to induce superagonistic signalling by a receptor according to claim 20 or 24, wherein said antibodies have been generated in a combinatorial antibody library.

31. Method of obtaining a superagonistic antibody as defined in any one of claims 19 or 22 to 24 comprising

(i) immunising an animal with a peptide comprising a sequence of length 5 to 20 amino acids which represents an extracellular membrane proximal region of the receptor and obtaining the antibody produced by the animal against said sequence, or

(ii) selecting an antibody from a combinatorial antibody library based on its ability to bind a peptide as defined in (i), and optionally

(iii) recombinantly expressing the antibody obtained in (i) or (ii).

32. Method according to any one of claims 1 to 9 or 25 to 31 further comprising formulating the identified modulator, obtained antibody or obtained agent into a pharmaceutical composition.

33. A peptide of length 5 to 20 amino acids comprising a sequence that binds to an antibody according to any one of claims 19 or 22 to 24.

34. Method of inducing superagonistic signalling by a receptor according to claim 20 or 24 on a cell surface comprising sterically inhibiting contact between a phosphatase of the cell and the receptor, excluding a method in which an antibody that binds only the C'-D loop of CD28 is used to sterically inhibit contact between CD28 and the phosphatase CD45.

35. Method of modulating the immune response of a patient comprising administering to the patient:

(i) a modulator identified by the method of any one of claims 1 to 9, or

(ii) an antibody or chimeric protein according to any one of claims 19 to 24 or which is obtained by the method of claim 30 or 31; or

(iii) an agent obtained by the method of any one of claims 25 to 29, or

(iv) a peptide that stimulates an antibody response in the patient, wherein the antibody response comprises an antibody according to any one of claims 19 or 22 to 24, or

(vii) a nucleic acid capable of expressing (i), (ii), (iii) or (iv).